REMARKS

In response to the restriction requirement set forth in the Office Action mailed June 3, 2005, Applicant hereby elects, with traversal, the invention of Group IV, which, as defined in the Office Action, is drawn to SEQ ID NO: 15. Claims 16-52, all of the claims presently in the application, are readable on that invention, meaning that they all cover either SEQ ID NO: 15 or a species of that peptide.

In case the Examiner has misunderstood Claims 16-32, Applicant wishes to point out that those claims are not directed to the administration of any *one* of the four listed peptides; rather, those claims call for the administration of a combination of *all four* of the listed peptides. While that was the case with those claims as originally worded, by virtue of the above amendment to Claims 16, 20-24, and 27, perhaps this is even more clear now.

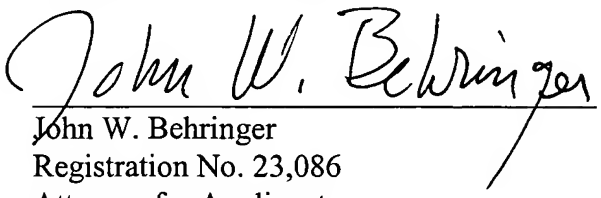
Newly added Claims 33-44, however, do *not* require the use of all four peptides in combination. They only require the administration of a peptide that comprises SEQ ID NO: 15.

Newly added Claims 45-52 are directed to the SEQ ID NO: 15 peptide per se.

Applicant is pleased to report favorable clinical data regarding the elected invention, as shown in the accompanying two reports (Exhibits A and B). These data will be presented in a Declaration Under Rule 132 by Dr. Ingebjørg Baksaas, who coordinated and monitored the study. The Declaration will be filed as soon as possible.

Applicant's undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 530-1010. All correspondence should continue to be directed to our address given below.

Respectfully submitted,



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Clinical phase II data

Applicant: Bionor Immuno AS, NO-3703 Skien, Norway
Sequences and claims according to Patent application no. XXXXXXXX, amended claim set DATE.

Introduction

A candidate immunotherapy "Vacc-4x" consists of four synthetic peptides corresponding to conserved domains of the HIV-1 p24 capsid protein. The four peptides are SEQ ID NO: 3, 6, 11 and 18. The bulk peptides were synthesised as described in the application (Patent application no. 00805735.4).
Data presented here for SEQ ID NO 18 are presented to document properties of sequences described by SEQ ID NO: 15.

Materials and Methods

Study design and immunisation schedule

The open, prospective randomised phase II clinical study included 38 healthy HIV positive patients stable on antiretroviral therapy (HAART) for at least 6 months, aged over 18 years, HIV positive >1 year, in generally good health, HIV RNA <400 copies/ml with nadir and actual CD4+ T cell counts >100 and >300 * 10^6/l, respectively. Exclusion criteria were pre-study AIDS-defining clinical events, acute primary HIV-infection or concurrent malignant disease, chronic active infection or immune-suppressive therapy.
The 52 week long study was divided into two periods of 26 weeks. The first half with immunisations given on HAART, the second half of the study was a treatment interruption study without immunisations. Patients were taken off HAART for 4 weeks followed by 8 weeks back on treatment before HAART again was removed for 12 weeks.

The patients were randomised into two dose level groups: Low dose (LD; n=18) and high dose (HD; n=20) receiving intradermally 0.1 ml peptide composition containing either 0.4 mg or 1.2 mg peptides per immunisation, respectively. The patients received a total of 10 intradermal injections at weeks 1, 2, 3, 4, 6, 12, 13, 21, 22 and 26. Equally for both dosage groups 30 µg recombinant human Granulocyte macrophage colony stimulating factor (GM-CSF) (Leucomax®, Schering-Plough), in 0.1 ml water, was given as a local intradermal adjuvant 15 minutes prior to Vacc-4x injection.The study was approved by the Norwegian Medicines Agency and the Regional Ethics Committee. Written informed consent was obtained from each patient before the study.

Experimental methods

Delayed type hypersensitivity (DTH) tests with the peptide composition where performed in three concentrations, 0.02, 0.1 and 0.4 mg peptides in 0.1 ml water, at 8 occasions during the study (weeks 1, 3, 6, 12, 21, 26, 38 and 52). At 2 occasions (week 18 and 24) four DTH-tests (called "single-DTH") were performed with each of the four peptides SEQ ID NO: 3, 6, 11 and 18 alone (0.1 mg in 0.1 ml). All DTH tests where injected, at the left and the right forearms, without adjuvant. Induration areas were measured 48 h after intradermal injection of peptides (Type IV DTH). Areas were calculated as πr^2 where r denotes ½ of the average of the shortest and longest diameter. Diameters were measured with an uncertainty of less than 0.5 mm. The measurements were performed by the same study nurse through out the study. DTH induration was considered

positive when the area was > 10 mm^2, which was the maximum induration observed with water alone.

Antigens and antibodies

Staphylococcal enterotoxin B (SEB) (Sigma) was used as positive T cell activation control at 0.5 - 1 µg/ml in the T cell proliferative assay. Cytomegalovirus (CMV) lysate (Dade Behring) was used at a 1/200 dilution of the stock solution from the supplier. Recombinant p24 protein was obtained from National Institute for Biological Standards and Control, UK. The peptides and the corresponding native peptides were manufactured by Isopharma (Amersham Health, Norway) and used at 2.5 µg/ml for each peptide.

Analysis of T cell proliferation

T cell proliferative responses were measured in peripheral blood mononuclear cells (PBMC) obtained from CPT tubes and pulsed with the fluorescent cytosol stain carboxyfluorescein diacetate succimidylester (CFSE) at 4µM for 5 min in protein free PBS. Cells were cultured with antigen (Ag) in duplicate wells for 7 days in serum-free AIM-V medium (Gibco, UK) with 0.1% albumin in 96-wells tissue-culture plates (Nunc), 10^5 cells/well at 37°C in 5% CO_2. Cells were stained with mAbs diluted in PBS with 0.5% EDTA, 0.05 % sodium-azid, 0.1% albumin and 8.3 mg/ml human polyclonal IgG (Octapharma, Norway) at 20°C for 20 minutes in dark. The cells were subsequently washed and finally fixed in 1% paraformaldehyde in PBS with 0.1% albumin prior to flow analysis.

Results for SEQ ID NO: 18

Median values of single peptide DTH induration areas at week 18 and 24 are shown in figure 1. The data show that the peptide is immunogenic.

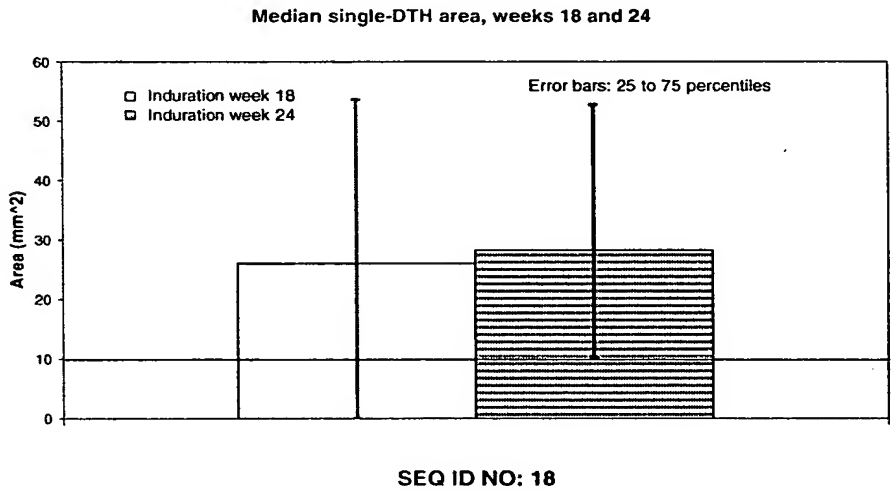

Figure 1: Median DTH induration area for single-peptide SEQ ID NO: 18 at week 18 and 24. Error bars indicate 25 and 75 percentiles.

Proliferative responses to each of the peptide (SEQ ID NO: 18) was detected at weeks 12-52. Data measured at week 52 is shown in Fig. 2. Proliferative responses to native equivalents (HIV-1 clade B peptides as described in the patent application) is also shown. The peptide SEQ ID NO: 18 which is from the group SEQ ID NO: 15 give a very strong response.

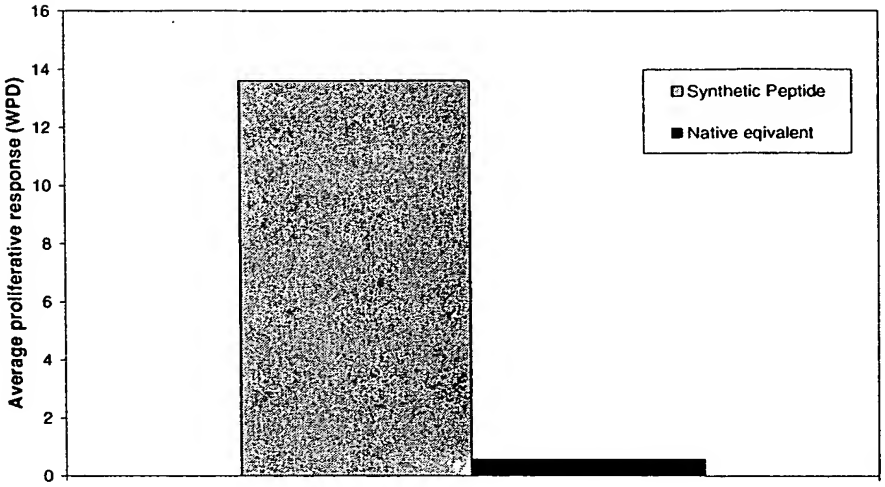

Figure 2: Proliferative CD3+ T cell responses (weighed percent divided (WPD)) above background for synthetic peptide SEQ ID NO: 18 and native equivalent (HIV- 1 clade B p24 peptide) at week 52(end of study). Higher proliferation was observed compared to the native equivalent.

Conclusions

The data show that the peptide SEQ ID NO : 18 is immunogenic, and show better immune responses than the native equivalent. **The data in particular show that a peptide from SEQ ID NO: 15 can give strong immune responses both in vitro and in vivo.**

Clinical phase II data

Applicant: Bionor Immuno AS, NO-3703 Skien, Norway
Sequences and claims according to Patent application no. XXXXXXXX, amended claim set DATE.

Introduction

A candidate immunotherapy "Vacc-4x" consists of four synthetic peptides corresponding to conserved domains of the HIV-1 p24 capsid protein. The four peptides are SEQ ID NO: 3, 6, 11 and 18. The bulk peptides were synthesised as described in the application (Patent application no. 00805735.4).
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CD4+ T-lymphocyte counts were measured by flow cytometry after direct staining of EDTA peripheral blood with the TriTEST reagent kit (Becton Dickinson, Biosciences; San Diego, USA (BD)). HIV RNA was monitored by the COBAS Amplicor HIV-1 monitor test (Roche) with a detection limit of 50 copies/ml. All blood samples were collected prior to immunisation.
Delayed type hypersensitivity (DTH) tests with the peptide composition where performed in three concentrations, 0.02, 0.1 and 0.4 mg peptides in 0.1 ml water, at 8 occasions during the study (weeks 1, 3, 6, 12, 21, 26, 38 and 52). At 2 occasions (week 18 and 24) four DTH-tests (called "single-DTH" hereafter) were performed with each of the four peptides SEQ ID NO: 3, 6, 11 and 18 alone (0.1 mg in 0.1 ml). All DTH tests where injected, at the left and the right forearms, without

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Comparison with Placebo group

HIV immunotherapy aims to provide prolonged drug-free periods to alleviate toxic side effects of HAART. The phase II clinical trial of the immunotherapy candidate Vacc-4x concluded with an interruption of HAART. Patients remained HAART-free for a median of 70 weeks. A retrospective cross-study comparative analysis was made between Vacc-4x patients and comparable patients from the Athena cohort (Wit FWNM, Blanckenberg DH, Brinkman K, Prins JM, van der Ende M, Schneider MME, Mulder J-W, de Wolf F, Lange JMA. Safety of long-term interruption of successful anti-retroviral therapy: the ATHENA cohort study. AIDS **2005**; 19:345-8.). The Athena patients interrupted HAART without receiving Vacc-4x.

Results

No serious adverse events were reported during the period of immunisation. Most patients reported mild local reactions at the site of injection. No changes in performance status, vital signs or safety laboratory parameters were observed. HIV RNA (Viral load) and CD4+ T cell counts remained stable throughout the immunisation period. (Kran A-MB, Sørensen B, Nyhus J, Sommerfelt M, Baksaas I, Bruun JN, Kvale D. AIDS 2004 18: 1875-1883.) This was also in line with an earlier safety study on the same peptide composition (Asjo B, Stavang H, Sorensen B, Baksaas I, Nyhus J, Langeland N. AIDS Res Hum Retroviruses 2002, 18:1357-1365). When HAART were stopped, in the second part of the study, viral rebound was observed in most patients, but viral levels stabilised and were controlled by the patients. An association was found between immunological response to the peptides and degree of viral control. This is shown in figures (5-7) and further explained in (Kran A-MB, Sommerfelt MA, Sørensen B, Nyhus J, Baksaas I, Bruun JN, Kvale D. Reduced viral burden amongst high responder patients following HIV-1 p24 peptide-based therapeutic immunization. Vaccine **2005**; In Press.). The patients were given the option to stay off HAART at

the end of the study. Their CD4, CD8 and viral load profiles have been followed until they have returned to HAART therapy.

Delayed type hypersensitivity reactions to peptide composition

Figure 1 shows median values (n=38) of DTH reactions to the peptide composition, at the three concentrations (0.4, 0.1 and 0.02 mg in 0.1 ml). All patients were tested for their reactivity to the peptide composition at week 1 (baseline), the same week the injections started. A positive DTH reaction was defined as a skin induration area greater than 10 mm^2. At week 1 a positive DTH induration area was observed in 6 (16%) of the patients regarding the highest DTH dose (0.4 mg) compared to 5 (13%) using the lower DTH doses (0.1 and 0.02 mg). Those patients that had a (weak) reaction at week 1 developed stronger reactions in the course of the study. The patients typically had their first DTH reactions at week 3 or 6. At week 12 the median DTH areas stabilized and remained roughly constant until week 52. Overall, larger DTH responses developed at all time points compared with baseline ($p<0.001$). At the end of the immunization period, week 26, Thirty five (90%) of the patients had positive DTH reactions.

The data shows that the peptide composition Vacc-4x is immunogenic even in chronically infected patients with dysfunctional immune responses (HIV-positive).

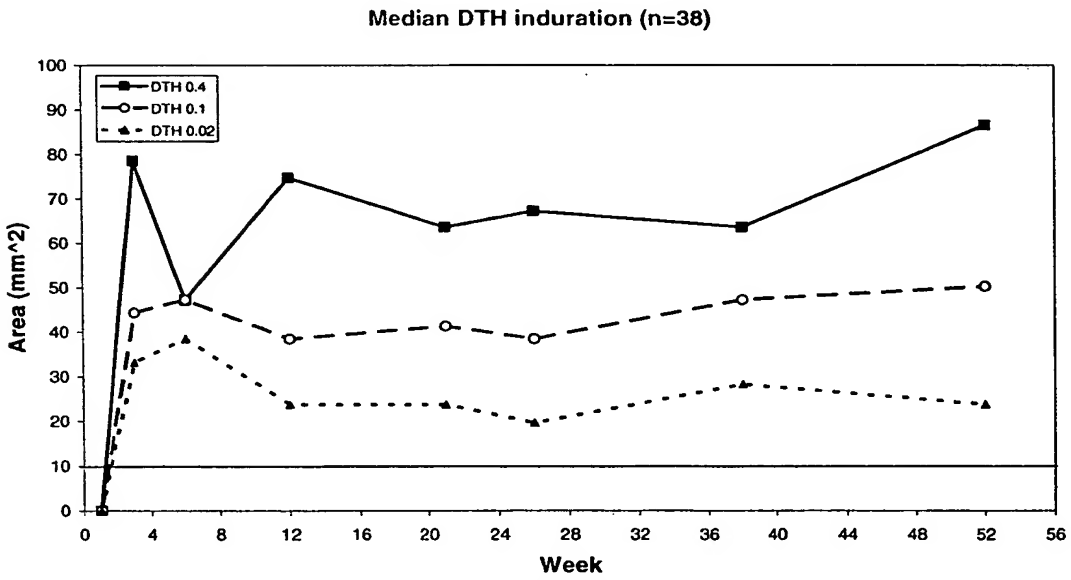

Figure 1: Median DTH area (mm^2) at weeks 1-52 for the 38 patients, measured at three peptides concentrations (0.4, 0.1 and 0.02 mg in 0.1 ml water).

DTH reactions to single peptides

The peptide composition consists of four peptides named SEQ ID NO: 3, 6, 11 and 18. DTH-tests were performed at weeks 18 and 24 for each of these peptides, using 0.1 mg peptide in 0.1 ml water. Median values of single peptide DTH induration areas at week 18 are shown in figure 2. Similar data was observed in week 24. All peptides show induration areas of comparable size. Statistically there are no significant size differences between the peptides, meaning that they are similarly good at inducing in vivo immune responses (DTH induration).
The data show that all peptides are immunogenic, and that the DTH reactions measured for the peptide composition (figure 1) contains contributions from all of the four peptides.

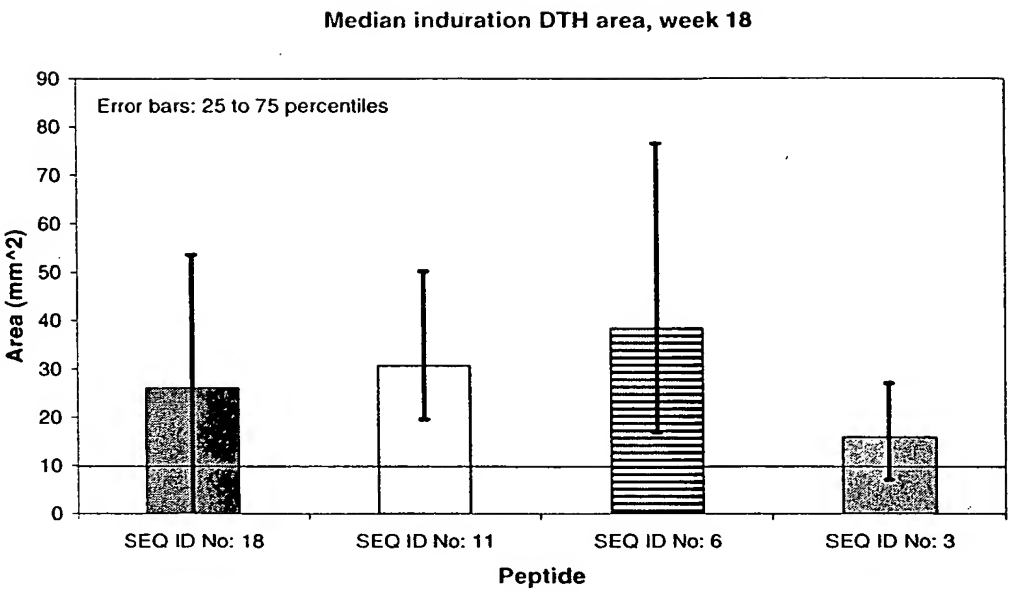

Figure 2: Median DTH induration area for single-peptides (SEQ ID NO: 3, 6, 11 and 18) at week 18. Error bars indicate 25 and 75 percentiles.

T-cell proliferation

Peptide-specific proliferative responses were detected in 80% of the patients from week 12. These responses were higher than in a group of 16 non-immunized control patients ($p<0.01$), where only 5 (31%) of 16 had very weak, but detectable responses. In contrast, no such differences were found between control patients and immunized patients in response to CMV. Peptide-composition-specific proliferation was higher than controls in both the LD ($p=0.05$) and HD group ($p<0.001$) at the end of the immunisation period (week 26), as shown in Fig. 3. Similar data was seen for the CD4+ and CD8+ subsets, as well as at other time points. When looking for associations between proliferation and DTH area, we found that patients with the most extensive DTHs (upper quartile) also had the highest CD4+ ($p= 0.03$) and CD8+ ($p=0.02$) T cell proliferative responses. Moreover, patients with the lowest proliferative T cell responses (lower quartile) had smaller DTH infiltrates ($p=0.06$), particularly within the LD group ($p=0.03$). The data show that the observed T-cell proliferation is due to immunisation with the peptide composition.

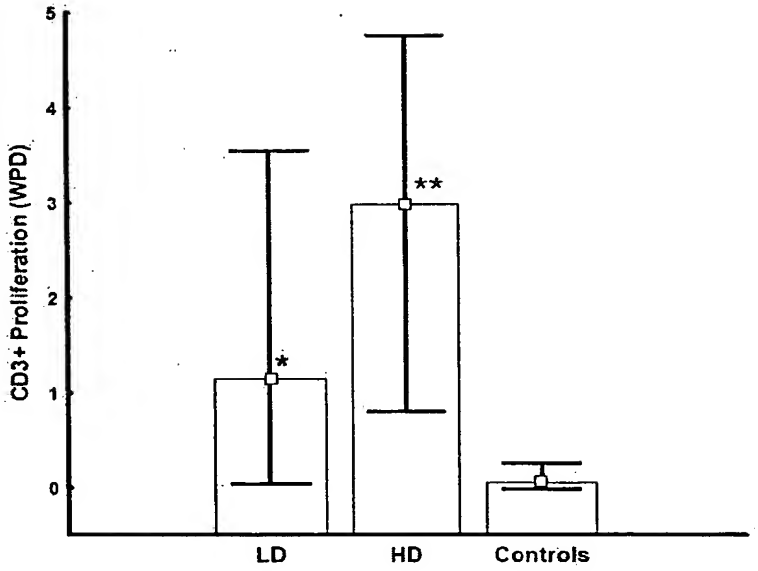

*Figure 3: Proliferative CD3+ T cell responses (weighed percent divided (WPD)), above background at week 26 in the two dosage arms (LD, HD) and in matched controls. Significantly higher proliferation in vaccinees than in unvaccinated controls are marked with * ($p=0.05$) or ** ($p<0.001$). Similar results were found in the CD8+ and CD4+ T cell subsets. Medians and 25-75 percentile ranges indicated.*

T-cell proliferative responses to single peptides

Proliferative responses to each of the peptides (SEQ ID NO: 3, 6, 11 and 18) was detected at weeks 12-52. Data measured at week 52 is shown in Fig. 4. Proliferative responses to native equivalents (HIV-1 clade B peptides as described in the patent application) is also shown. The synthetic peptides all give a stronger proliferative response compared to their native equivalents. **Particularly the peptide SEQ ID NO: 18 which I s from the group SEQ ID NO: 15 give a very strong response.**

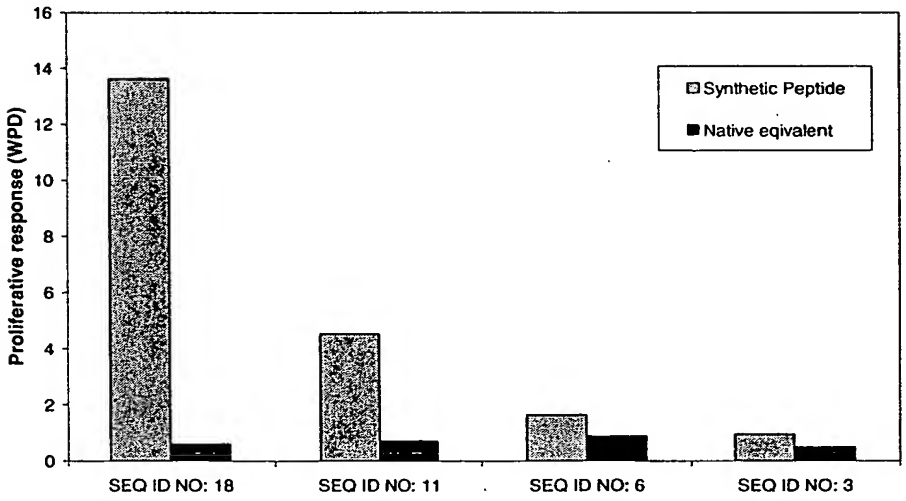

Figure 4: Proliferative CD3+ T cell responses (weighed percent divided (WPD)) above background for synthetic peptides (SEQ ID NO: 3, 6, 11 and 18) and native equivalents (HIV- 1 clade B p24 peptides) at week 52. Higher proliferation was observed for all synthetic peptides compared to their native equivalents.

Virus control

90% of the patients reacted to the peptide composition *in vivo*. As indicated in figs 2 and 4 the HD group had greater immunological responses to the immunotherapy, however, some of the LD patients also had quite strong immunological responses to the immunotherapy. Therefore independent of dosage groups, Vacc-4x high-responders (Hi) were defined by having DTH responses at week 38 above upper quartile (n=10).
Median Vacc-4x responders (Med) had DTH responses within the 25-75 percentiles (n=17) and DTH low-responders (Lo) lay within the lower quartile (n=11). These groups reflected better the *in vivo* immunological responsiveness to the peptide composition as shown below.

There were no significant differences between DTH Hi and Lo responders in nadir CD4, time from seroconversion, time on HAART, pre-HAART HIV RNA, or pre-HAART or pre-immunization CD4- and CD8-counts. Moreover, at study baseline, no differences in DTH areas or p24-specific responses were detected.

DTH High-responders developed generally greater proliferative responses than the DTH low-responders, both to the peptide composition (p=0.09) and p24, as shown in Figs. 5 and 6. Peptide-composition- and p24-specific CD3+ T cell-responses at week 38 (after the first STI) are shown. The data show that immunising with the peptide composition lead to cross reactions that increase proliferative response to HIV-1 p24

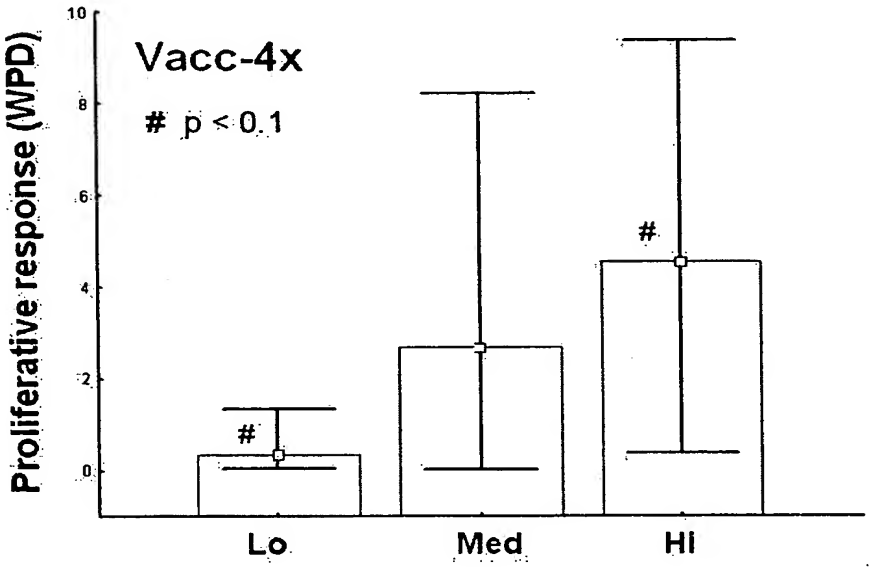

Figure 5: Proliferative responses to Vacc-4x at week 38 shown for three groups (Lo, Med and Hi) according to in vivo (DTH) responsiveness.

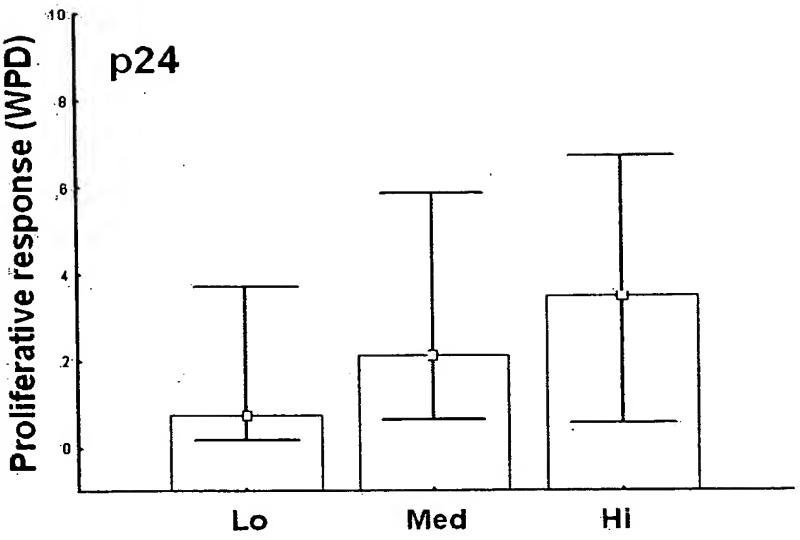

Figure 6: Proliferative responses (weighed percent divided (WPD)) to recombinant HIV-1 p24 protein at week 38 shown for three groups (Lo, Med and Hi) according to in vivo (DTH) responsiveness.

High-responders had significantly lower viral load at week 52 compared to Low-responders both in terms of actual HIV RNA (median 39 000 versus 130 000 copies/ml, p=0.08) and ratio of actual HIV-RNA/pre-HAART viral load (median ratio 0.58 versus 1.30, p=0.04), as shown in Fig. 7. No differences in preHAART HIV RNA were found between these groups as seen in Fig. 8.

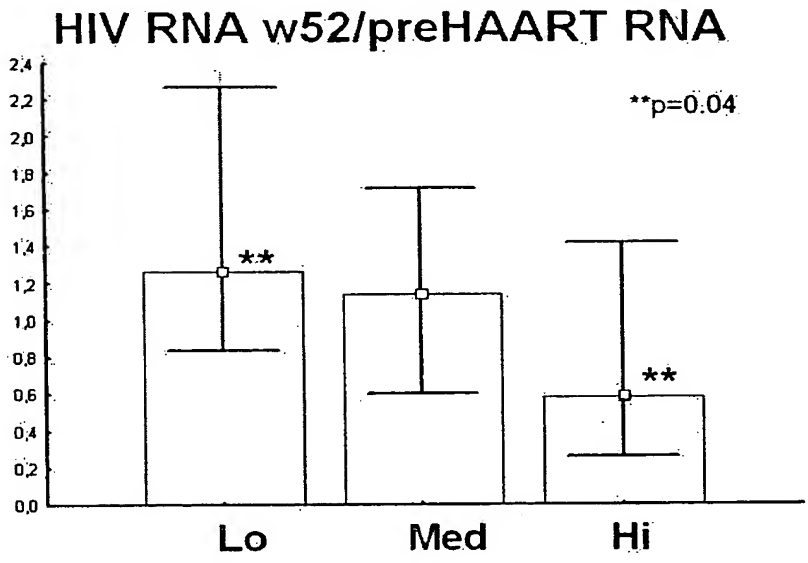

Figure 7: HIV RNA at week 52 compared to preHAART values. Significantly lower viral levels was observed in the Hi group than the Lo group (p=0.04)

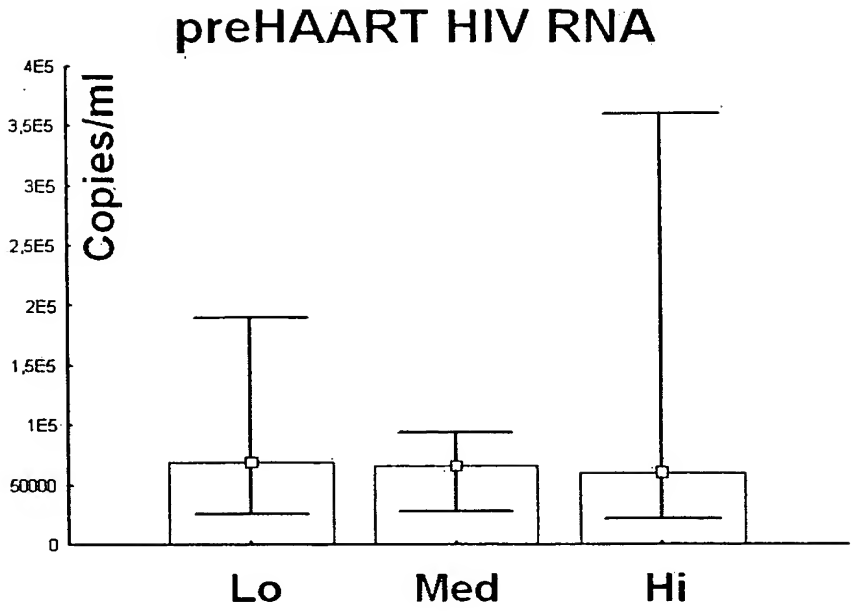

Figure 8: preHAART HIV RNA for the patients according to DTH responsiveness. No difference is seen between the three groups.

CD4-counts at the end of the study were also associated with DTH response, but not significantly so. DTH high-responders seemed to have slightly higher CD4-counts at the end of the study, whereas no such associations were detected pre-HAART or pre-study.

All in all responsiveness to the peptide composition Vacc-4x is associated with improved viral control for the immunised patients.

Comparison with non-immunized patients

The Vacc-4x group showed a significantly slower decline in mean CD4+ T-cell counts (p=0.0005) and a small but significant delay in viral rebound (p=0.02). The Vacc-4x patients stayed off HAART significantly longer than the Athena patients (Median 70 weeks compared to 17 weeks). Data are shown in figures. 9 and 10

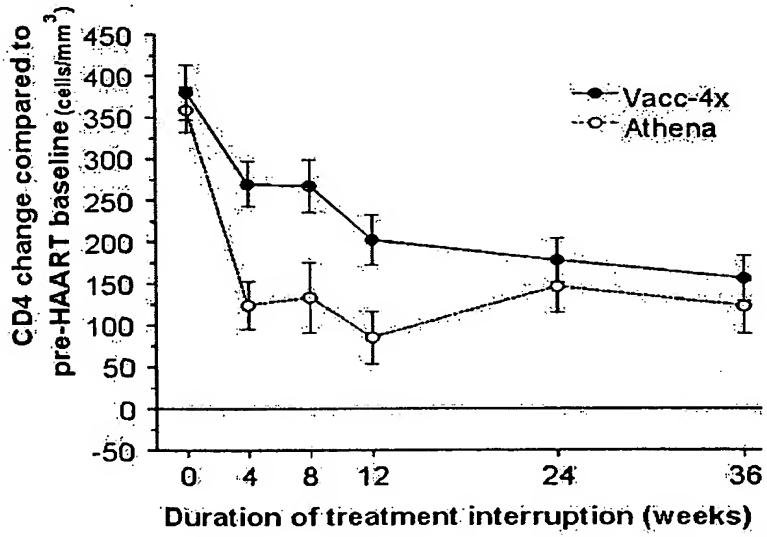

Figure 9: Changes in CD4$^+$ T-cell counts after interruption of HAART. The changes in CD4$^+$ T-cell counts are compared to the pre-HAART levels (the horizontal line). There is a significantly slower decline in CD4 T-cells for the Vacc-4x group (p=0.0005) than the Athena-group.

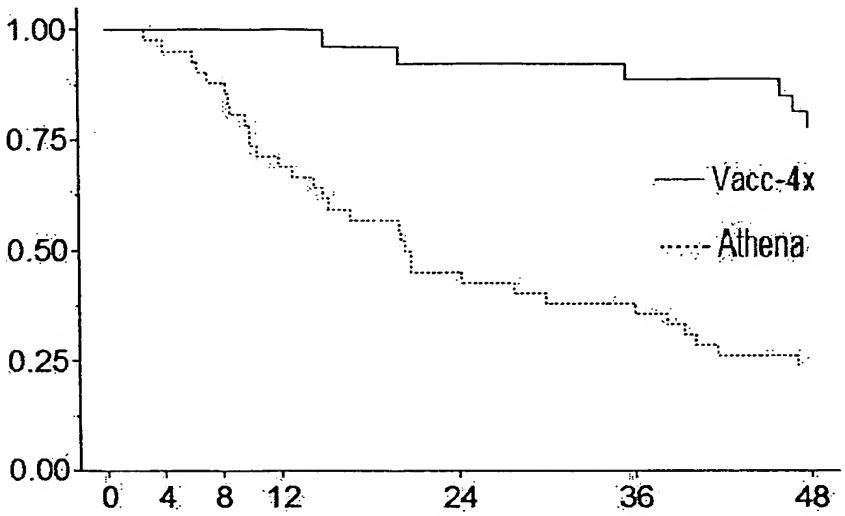

Figure 10. Kaplan-Mayer plot showing percentage of patients remaining off HAART in the two groups.

Conclusions

The data show that the peptide composition Vacc-4x is immunogenic even in patients with dysfunctional immune responses, and the patients could safely be taken off HAART.
The data show that the peptides (SEQ ID NO : 3, 6, 11 and 18) are all immunogenic, and that the DTH reactions measured for the peptide composition contains contributions from all of the four peptides. The data further show that the observed T-cell proliferation is due to immunisation with the peptides and that these synthetic peptides all give a stronger proliferative response than their native equivalents. Immunising with the peptide composition lead to cross reactions that increased proliferative responses to HIV-1 p24. All in all responsiveness to the peptide composition is associated with improved viral control for the immunised patients. **The data in particular show that a peptide from SEQ ID NO: 15 can give strong immune responses both in vitro and in vivo, and that this can be coupled to clinical benefit for the patients.**